RELATIVE VIABILITY OF B. COLI AND B. AEROGENES TYPES IN WATER

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The division of the so-called "colon group" of aerobic gas-forming bacilli into two distinct types, which we owe to the researches of Rogers and his co-workers [1, 3, 6] has opened a wide range of questions, of practical as well as theoretical importance. It seems clear that the B. coli type (low gas ratio, methyl-red positive, Voges-Proskauer negative) is the predominant form in feces while the B. aerogenes type (high gas ratio, methyl-red negative, Voges-Proskauer positive) is apparently the characteristic form found on grains and grasses. The thought has therefore naturally suggested itself that the differentiation between these two types of gas-forming organisms might prove of practical value in sanitary water examinations for distinguishing between surface wash from areas of normal vegetation and those which receive specific fecal pollution. This question of the relative distribution of B. coli and B. aerogenes types in waters of different origin have been discussed by us in another paper.[10]

Aside from this question of the original source of the organisms there is another possibility which might prove significant—the possibility of a difference in the relative viability of the two types in water.

Clemesha,[2] in his very suggestive study of the distribution of colon group organisms in tropical waters, found that in grossly and recently polluted water, B. lactis-aerogenes is a rare organism, but that usually within a period of about 5-15 days after pollution in lakes, this organism becomes extremely common. B. cloacae, another representative of the methyl-red negative type, is one of the most resistant organisms in water. This organism seems to be able to multiply in waters and is the most abundant lactose fermenter in dry weather. Rogers, Clark

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[1] Rogers, Clark, and Evans: Jour. Infect. Dis., 1914, 15, p. 100. Clark and Lubs: Jour. Infect. Dis., 1915, 17, p. 160.

[2] Bacteriology of Surface Waters in the Tropics, 1912.

[3] Jour. Bacteriol., 1916, 1, p. 82

[6] Jour. Infect. Dis., 1915, 17, p. 137.

and Lubs[3] are of the opinion that B. aerogenes is more resistant to unfavorable conditions than the other more numerous fecal types, and should therefore survive after the latter have disappeared.

We find on study of the data of Houston[4] that of the lactose-fermenting coliform bacteria isolated from 3 types of water the following percentages gave a positive Voges-Proskauer reaction (characteristic of the aerogenes group):

From raw water,	12.9%	of 243 cultures
From stored water,	5.3%	of 133 cultures
From stored and filtered water,	3.2%	of 156 cultures

These figures seem to contradict the idea that the B. aerogenes group is the resistant one. Rogers,[5] on the other hand, in several studies of this question, did find a difference in resistance of the 2 types. Thus, in water artificially infected with feces and held at 20 C there was a gradual change in the ratio of the 2 types until at the end of 9 months there were 39 B. aerogenes to 1 B. coli. In sewage held in running water there was a rapid decrease of colon group bacteria which was more evident with the B. coli type. At the beginning there were about 3 times as many B. coli as B. aerogenes, but after 7 days there were slightly more B. aerogenes than B. coli.

EXPERIMENTAL PROCEDURE

The following experiments were undertaken in order to throw further light on this question of the relative viability of the two types of gas forming organisms.

Ten glass bottles each of 8 liter capacity, and 1 of 16 liters capacity were used. These were all thoroughly washed. Bottles VII to XI were autoclaved prior to filling with water, the others received no further treatment. The bottles were then filled with ordinary tap water to the 7-liter mark—the large bottle to the 14-liter mark. The bottles were stoppered and well shaken, and samples of the water were examined for total count and the presence of gas and acid formers in lactose broth. The counts were always low and gas and acid formers were in no case present.

Each bottle was then inoculated with a loopful of each of 3 different agar slants of B. coli and 3 different cultures of B. aerogenes. The 14-liter bottle, Number VI, received twice the above amount of inoculum. In the case of Bottles I to IV, the loops of culture were shaken directly into the water, and the bottles then thoroughly agitated. In the case of Bottles V to XI, the loops were shaken into 10 c c of dilution water and the resulting suspension

[4] Seventh Research Report to Metr. Water Board, London, 1911.
[5] Bact. Abstr., 1917, 2, p. 56.

thoroughly shaken before being added to the bottles. Promptly after inoculation samples from each bottle were examined in suitable dilutions for

1. Gas production in lactose broth.
2. Acid production on azolitmin-lactose-agar plates.
3. Total count on plain agar plates at 37 C.

Gas production and total count were noted at the end of 48 hours.

The acid colonies were noted from the 12th hour on to the 24th. At the end of 24 hours, 20-25 consecutive acid colonies were picked off and inoculated into the methyl-red medium of Clark and Lubs.[6] The reason for beginning the observation of the plates by the 12th hour was the suspicion that the B. aerogenes type might 'revert' too soon to an alkaline reaction and thus escape detection at the end of 24 hours. Such an early reversion has not, however, been observed very often in this study.

After taking the first set of samples the bottles were set aside in the laboratory and successive samples were taken at regular intervals up to 60 days, the procedure in each test being the same as that described above. The whole work extended from December, 1916, to April, 1917, and the experiments were made in 5 series, begun at different times, 2 bottles being run in each series except the last, which included 3 bottles. Series 1 (Bottles I and II) was stored in a dark closet, the temperature of which varied from about 5 C.-15 C. during the experiment. All the other bottles were stored beneath a table in the laboratory. No attempt was made to exclude diffuse light from these bottles and the temperature varied between 10 and 20 C.

The criterion used for differentiating the B. coli and B. aerogenes types isolated from the stored water was the limiting hydrogen ion concentration attained in the Clark and Lubs medium. This medium, in our experiments, was made up with Difco peptone instead of Witte's. Ordinarily this might introduce some confusion in the test, because some of the organisms of the colon-aerogenes group do not behave alike in mediums made with different peptones. It is suspected that the reason for this difference in behavior lies in the buffer action of the two mediums as well as in the utilizability of the nitrogenous portions.

The particular cultures of B. coli and B. aerogenes used in these tests had all shown a marked stability in the production of a final P_H value in the methyl-red medium made up with Difco peptone. This final P_H was determined by the colorimetric method on 6-8 different occasions for these cultures, at intervals several months apart, and in different lots of the medium. At no time was there any marked variation in the final P_H. The values for the B. coli cultures ran from 4.6-5, while those for the B. aerogenes ran from 6.6-7. For the purposes of this experiment we were therefore safe in using the medium containing the Difco peptone.

The cultures obtained from the stored samples were incubated at 37 C. for 4 days, and then tested with methyl-red solution (0.1 gm. methyl-red dissolved in 300 c c alcohol and diluted to 500 c c with water). B. coli shows a brilliant red coloration while the B. aerogenes culture is yellow.

VIABILITY OF THE GROUP OF GAS FORMERS AS A WHOLE

Turning to our results, we have indicated in Table 1 the total numbers of bacteria per c.c. in the various bottles as tested. Bottles I-IV show a rise in numbers during the first few hours after inocula-

tion. We are somewhat doubtful whether there is any multiplication going on even during this short period. We are rather inclined to attribute the above result to the fact that in the first 2 series the loops of culture were added directly to the water instead of first being thoroughly distributed to break up clumps. In the succeeding series where a preliminary suspension was made in water, we did not find any rise in numbers over the initial count. In any case after this there was a steady decline to the end of the experiments. Bottle V exhibited an unusually fast decline in numbers. On investigation it was discovered that this bottle had for years held bichlorid solution, and apparently, the cleaning it received at our hands failed to remove all traces of the disinfectant.

TABLE 1

TOTAL BACTERIAL COUNT IN STORED WATER (BACTERIA PER C C)

	Series 1		Series 2		Series 3		Series 4		Series 5			Aver. of All
Bottles	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Bottles
Time												
0	224,000	161,000	172,000	178,000	125,000	78,000	935,000	877,000	235,000	228,000	225,000	313,000
4 hours	313,000	212,000										
6 hours			179,000	159,000	56,000	64,300	834,000	762,000				
1 day	256,000	194,000	133,000	108,000	20,800	29,700	609,000	610,000	138,000	155,000	180,000	221,000
3			66,700	33,300	1,670	29,000	493,000	466,000	135,000	138,000	132,000	166,000
5	20,700	21,000	2,800	5,600	0	22,000	31,000	36,600	16,000	16,000	24,000	17,800
10	2,700	3,250	990	3,900	0	850	3,000	1,700	4,000	3,300	4,000	2,500
15	1,630	2,600										
20	1,400	1,270	280	1,500		475	2,000	1,600	2,800	1,200	1,200	1,250
30	580	550	180	350		100	200	1,500	1,600	1,100	700	620
40	200	180	100	195		90	140	250	1,400	500	300	305
50	200	100	65	100		80	110	150	150	300	300	141
60	63	67	60	100		60	130	150	70	100	90	81

It is interesting to note that by the 10th day there was a 98 or 99% reduction in numbers in every bottle but one. This point is in agreement with other observations made in experiments comparable with ours. Thus, Houston[7] found a 99% reduction of B. typhi after 1 week of storage in bottles of raw river water. Clemesha also noted a marked diminution in numbers of colon bacilli on storage. Rogers[5] demonstrated a marked falling off in numbers of colon organisms when permeable sacs containing raw sewage were placed in running water for a period of 7 days. Rector and Daube[8] studying the longevity of B. coli in water, noted an initial increase in numbers and then a gradual decrease, with final disappearance of the organisms in about 50 days.

[7] First Research Report to Metr. Water Board, London, 1908.

[8] Bact. Abstr., 1917, 1, p. 57.

RELATIVE VIABILITY OF B. COLI AND B. AEROGENES

In Table 2 are given the percentages of B. coli (positive methyl-red organisms) found at the various periods when the bottles were sampled. It is to be remembered that we aimed at adding approximately equal numbers of B. coli and B. aerogenes to the water in the first place, and the first horizontal line of figures in this table shows how near we came to doing so. This is of course only suggestive, for we realize that when there are several hundred thousand bacteria in a c c it is hard even with all proper precautions to get 20-25 organisms that will be representative of the true relationship between the types present. The results of the various series run closely parallel, however, and we may reasonably conclude that the figures are near the actual truth.

TABLE 2

PER CENT B. COLI AMONG THE SURVIVING GAS FORMERS

	Series 1		Series 2		Series 3		Series 4		Series 5			Aver. All Observations
Bottles	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	
Time												
0	50	70	92	92	52	44	32	40	56	24	40	54
4 hours	65	50										
6 hours			92	92	60	40	44	60				
1 day	65	85	88	92	76	80	40	32	56	24	52	63
3 days			96	92	0	60	48	52	40	20	44	50
5 days	45	40	80	80		100	32	36	28	8	32	48
10 days	35	65	76	88		64	32	28	28	8	40	46
15 days	30	55										
20 days	40	65	100	92		48	0	32	12	12	40	44
30 days	15	50	92	92		32	24	28	12	8	40	39
40 days	25	50	92	96		32	16	32	8	0	36	39
50 days	0	40	96	96		20	12	28	8	4	8	31
60 days	5	20	96	100		17	16	28	0	0	4	29

The first point to be noted in these experiments is that there was as a rule an initial increase in the percentage of the methyl-red positive types. This increase reached its maximum during the 1st day in 6 cases, on the 3rd day in 2 cases, on the 5th day in 1 case, on the 20th day in 1 case, and on the 60th day in 1 case.

In all but the last two instances cited (Bottles III and IV of Series 2) there then followed a relative decrease of the methyl-red positive (B. coli) type. In every bottle except the two just mentioned and one other (Bottle VI) the proportion of B. coli was less on the 5th day than at the beginning and after this period the ratio of B. coli to B. aerogenes (in every case except Bottles III and IV) continued to fall off fairly steadily till the end of the experiment.

The behavior of the organisms in Bottles III and IV was anomalous throughout. Instead of a fairly even initial distribution of B. aerogenes and B. coli at the beginning of the experiment these bottles contained in each case 92% of the latter type, which remained predominant to the end of the experiment. The apparent increases to 100% noted on two occasions were merely due to chance inequalities of distribution but the small number of B. aerogenes present did not in any case show an appreciable relative increase.

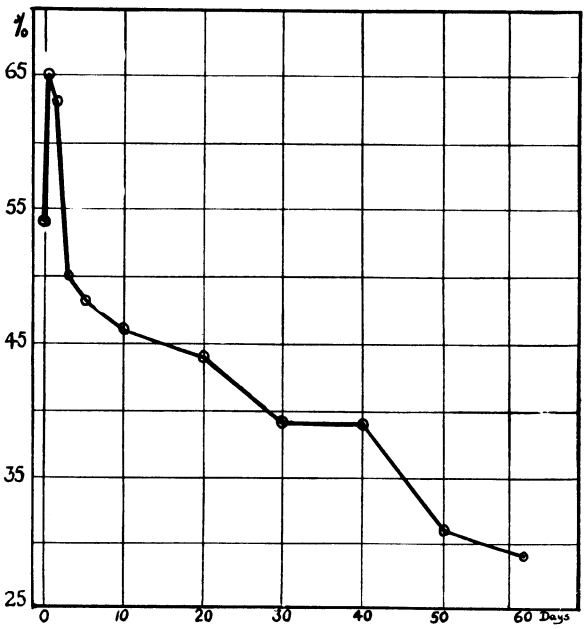

Chart 1.—Ratio of B. coli type to total gas-forming organisms after storage in water for various periods.

In general, however, the lesson of these experiments seems fairly clear. In the last column of Table 2 and in Chart 1 are given the general averages of all observations for each period (except for the periods of 4 hours, 6 hours and 15 days for which there were not sufficient records to be representative). The initial relative increase and marked subsequent decrease of the B. coli type are marked. From the two last columns of Tables 1 and 2 we have calculated the average number of organisms of each of the 2 types present at each period and from these data in turn we have computed the average numbers and

rate of reduction of each type. The results as shown in Table 3 and Chart 2 indicate that neither type followed a normal logarithmic rate of reduction, the rate in each case being very rapid at first and very gradual afterward. This is the sort of death curve which Chick[9] finds to be characteristic of old cultures containing organisms of widely differing vitality. Since the slants from which our bottles were seeded had been incubated for a week this is just what might have been expected.

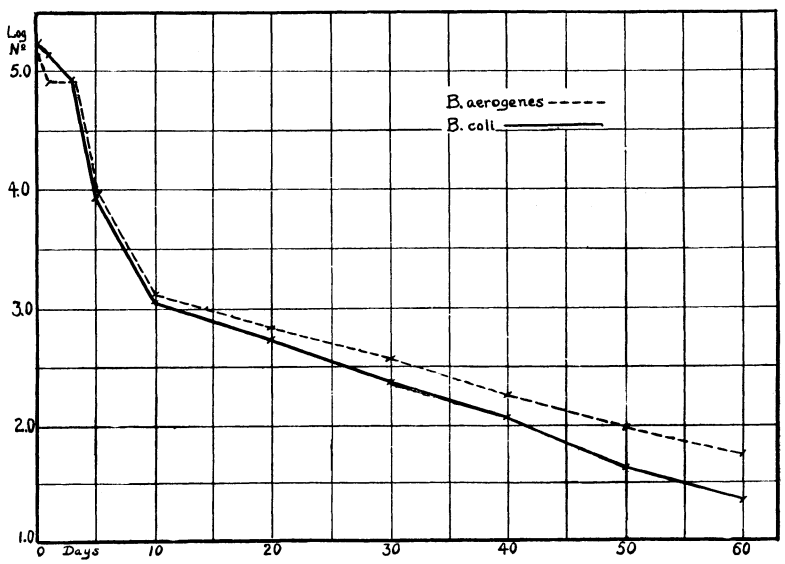

Chart 2.—Reduction of B. coli and B. aerogenes types on storage.

The differences in percentage reductions of the two types were at first quite marked, only 18% of the B. coli having disappeared after 1 day as against 43% of the B. aerogenes. By the 3rd day, however, the reduction of the B. coli was greater than that of the B. aerogenes and after the 5th day the per cent reduction of the 2 types was very nearly alike. If the very sharp drop in both curves at the beginning of storage was due as suggested above to the presence of old cells of low viability the difference between the 2 curves for this first period may have been due to a more rapid multiplication of B. aerogenes in the agar culture used for inoculation and the consequent presence of a larger proportion of susceptible cells.

[9] The Factors Conditioning the Velocity of Disinfection, Orig. Com., Eighth Int. Cong. Applied Chemistry, 26, p. 167.

On the other hand the persistence of B. aerogenes during the latter part of the storage period would appear to be most probably due to an inherently greater viability of this form under the conditions of the experiment. However, the actual differences were very slight as reference to Table 3 will show.

TABLE 3

REDUCTION OF B. COLI AND B. AEROGENES TYPES ON STORAGE

Time	B. Coli			B. Aerogenes		
	No. Present	Log. of No. Present	Reduction per Cent.	No. Present	Log. of No. Present	Reduction per Cent.
0	169,000	5.23	0	144,000	5.16	0
1 day	139,000	5.14	18.0	82,000	4.91	43.1
3 days	83,000	4.92	51.0	83,000	4.92	42.4
5 days	8,700	3.94	94.9	9,100	3.96	93.7
10 days	1,150	3.06	99.3	1,350	3.13	99.1
20 days	550	2.74	99.7	700	2.85	99.6
30 days	242	2.38	99.8	378	2.58	99.7
40 days	119	2.08	99.9	186	2.27	99.9
50 days	44	1.64	99.9	97	1.99	99.9
60 days	23	1.36	99.9	58	1.76	99.9

CONCLUSIONS

In general we may summarize as follows: when a mixture of gas-forming organisms of the B. coli and B. aerogenes types was stored in water under the conditions of these experiments:

All the gas-forming organisms present died off rather rapidly so that in general 98-99% had disappeared by the 10th day.

The B. aerogenes type decreased more rapidly than the B. coli type during the first 24 hours. After that period, however, the B. coli type died off more rapidly than the B. aerogenes type so that while 54% of all gas-forming organisms were of the B. coli type at the beginning of the experiments, this percentage fell to 29 after 60 days.

So far as the results after the 1st day of storage are concerned, our results confirm those of Clemesha and Rogers rather than those of Houston. Studies previously reported[10] indicate that a high proportion of the B. aerogenes type is not, as a matter of actual experience, common even in stored waters. In cases where a high proportion of this sort of gas former is actually found, however, it may apparently be due either to the fact that the gas formers present have come from grains rather than from fecal sources, or to a long period of storage which would tend to increase the relative frequency of the B. aerogenes as compared with the B. coli type.

[10] Winslow and Cohen: Jour. Infect. Dis., 1918,